§312.23(8)(b)(ii)(2) Subchronic Toxicity in Rabbits

A study of TRODAT's effects on rabbits was performed by the Toxicology Research Center of the State University of New York at Buffalo. Their report, dated 01 July 1997, has been attached as an appendix in PDF format.

The Toxicology Research Center was commissioned to perform the study according to their standard operating procedures. The test material consisted of [Tc-99]TRODAT-1 that was manufactured according to the SOPs specified in the application for human use. The [Tc-99m] was allowed to decay to [Tc-99] for at least 10 half-lives before the compound was shipped out of state.

The research subjects consisted of 14 laboratory rabbits, 7 of whom were administered TRODAT and 7 of whom were given a placebo. TRODAT or placebo was injected intravenously through a vein in an ear.

The primary outcome measures included organ to body weight ratios and histopathological examinations. The findings indicate that there are no significant adverse effects of TRODAT on these outcome measures in animals who are subchronicly administered doses that are approximately 50 to 65 times higher than those which our human volunteers will be receiving.

Some animals became distressed during the intravenous administration of TRODAT as well as the control solution. Their behavior most probably reflected a response to pain. Our first 10 human subjects never spontaneously reported discomfort during the injection of the radiopharmaceutical, but when asked directly, about half the volunteers described a slight burning sensation that can probably be attributed to the propellant, which contains ethanol. The rabbits were given relatively large doses of cold TRODAT or the control solution, and proportionately large volumes of the propellant. The injections were fast boluses, while clinically, we push slowly. This probably explains why the animals became distressed, while our human subjects hardly noticed.

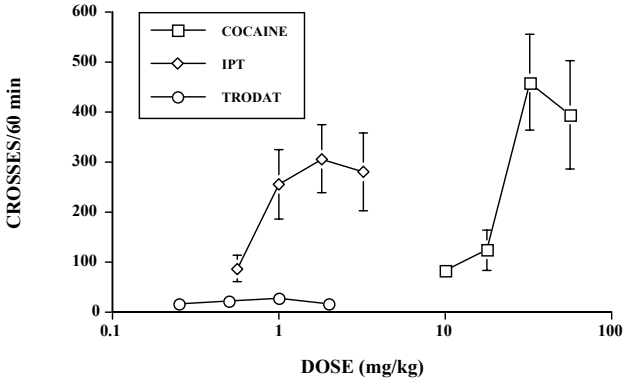
The increased white blood cell count on day nine can probably be explained by the same phenomenon. Universal responses to pain in mammals include a noradrenergically induced demargination of granulocytes which invariably lead to high, but clinically meaningless, white blood cell counts.

The same problem probably contributed to the femoral fracture sustained by one of the rabbits.

After receipt of the report, we started diluting TRODAT in 5 ml of sterile saline, which is added immediately before administration. About 280 subjects have now been asked directly if they felt a sensation upon injection. None have.

§312.23(8)(b)(ii)(3) Behavioral Toxicity Studies in Rats

The European Journal of Nuclear Medicine named a report by Kung et al. of pharmacology and toxicology as "Paper of the Year" in 1998. The behavioral effects of TRODAT were measured by Drs. Lucki and Essman of the Departments of Psychiatry and Pharmacology with a well established design.[i,ii] The effects of TRODAT, IPT, and cocaine on locomotive activity in rats were studied in 12 groups of 8 rats (200-225g) housed in groups of three in a temperature controlled environment. Food and water were available ad libitum for the duration of the experiment. Lights were controlled according to a 12 hr light:dark cycle (lights on 700 hrs). All experiments were conducted in the light portion of the animals cycle. Activity chambers (polycarbonate with wire mesh floors and stainless steel tops; 41 x 19 x 19 cm) were fitted with two photocells approximately 19 cm apart, 11 cm from the ends of the chamber. Interruptions of each photocell were monitored through a photocell controller connected to a microcomputer. All animals were acclimated to locomotor activity chambers approximately 4-5 days prior to the drug test. Each acclimation session consisted of a1 hour "acclimation" period and a 1 hour "test" period. On the day of drug administration, animals were placed in the chambers for a 1 hour acclimation session. The animals were then removed from the chambers, injected with a given dose of either cocaine or IPT (2 ml/kg; i.p.), and returned to the chambers for 1-3 hours, during which time their locomotor activity was monitored. Each group of animals was tested once. Locomotor activity was defined as the number of crosses (i.e., consecutive right-left or left-right photocell interruptions) an animal made over the course of a session. Data are presented as total locomotor activity over the first hour of testing (figure 1) or as activity per 5 min over a three hour time course for the maximally effective doses of cocaine and IPT and for the IPT vehicle (approximately 40% ethanol in water + acetic acid; figure 2). All comparisons were made by between-group ANOVAs followed by Dunnett's post-hoc tests. The figure at right shows the dose-response curves for the production of hyperactivity by IPT, TRODAT (unlabeled ligand plus [Tc-99] TRODAT) and cocaine. Both IPT and cocaine significantly increased locomotor activity (ANOVA: $F(4,33) = 5.379$; $p < .005$). IPT increased locomotion at 1.0, 1.8 and 3.2 mg/kg, with a maximum effect at 1.8 mg/kg. Cocaine was approximately 30-fold less potent, producing a significant increase in locomotion at 32 and 56 mg/kg. The dose-response experiment appeared to suggest that IPT was not as effective as cocaine in producing hyperlocomotion (compare maximum effects at 1.8 mg/kg IPT and 32 mg/kg cocaine). However, this difference was due to an increase in focused sniffing produced by IPT. Focused sniffing is a behavioral response characteristic of high doses of psychomotor stimulants. In contrast, TRODAT was not effective at producing significant changes in locomotor activity even at its maximum dose of 1.0 mg/kg. The ED_{50} for this hyperactivity effect was measured to be 0.8 mg/Kg ip for IPT, which



translates to a dose of about 56 mg (12.5 x 10-5 mole) ip for humans. In other words, the dose needed to induce hyperactivity is about one million times higher than the dose that is used during imaging studies with IPT (42 x 10-12mole). The next figure at right shows the time activity curves for the production of hyperactivity by 1.8 mg/kg of IPT, 1.0 mg/kg of TRODAT (ligand plus [Tc-99] TRODAT) and 32 mg/kg of cocaine, i.e., at their highest or maximum dose levels. Both IPT and cocaine increased locomotor activity above vehicle control levels over the first hour. The subsequent decrease in locomotor effects with IPT reflect the onset of focused sniffing, which effectively competes with locomotor activity in this behavioral paradigm. TRODAT was clearly less pharmacologically active than either IPT or cocaine. It follows that tracer doses of TRODAT are unlikely to cause any pharmacological effects in humans who participate in neuroimaging studies. This conclusion has been supported by the lack of an observable pharmacological effect during SPECT imaging studies in non-human primates ($n \approx 50$) using 10-20 mCi of [Tc-99m]TRODAT. It is consistent with the observation that tropane derivatives with specific binding to dopamine reuptake sites, such as [I-123]CIT (or RTI-55), and [C-11]CFT, (WIN35,428), have been used in humans successfully without any side effects (Frost et al. 1993).

§312.23(8)(b)(ii)(4)(a) Acute Cardiovascular Effects of High Dose TRODAT in a Non-Human Primate

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Co-Investigators: Steven Kushner, Karl Plössl, and Hank F. Kung, Ph.D.
Consultant: Luis I. Araujo, M.D., Chief of Nuclear Cardiology

Date of Study: 27 September 1996

Location of Study: Laboratory 114, Donner Building, Hospital of the University of Pennsylvania

Duration of the Study: 2.0 hours

Background: TRODAT is a new, Tc-99m labeled radiopharmaceutical for imaging the CNS dopamine transporters with SPECT. Dopamine transporter concentrations are exquisitely sensitive markers for the diagnosis of several clinically important neurodegenerative diseases. Currently, transporter ligands are only available as PET or [I-123] SPECT tracers. Successfully developing a Tc-99m labeled imaging agent would allow transporter concentration measurements to be made in conventional medical settings.

Rational for this type of toxicology study: TRODAT is a tropane analog of cocaine. Cocaine is known to have several clinically significant cardiovascular effects. These effects have a characteristic signature on EKG recordings.[iii,iv] It is not yet known whether TRODAT has an effect on the heart when given in the doses that will be used diagnostically.

Purpose: to determine whether the intravenous administration of TRODAT effects cardiovascular function in non-human primates.

Methods: A healthy, middle aged female baboon weighing 13 kg was anesthetized with 1.5 ml of ketamine HCl. Vital signs were monitored continuously with an automated pulse detector and blood pressure cuff placed on a thigh (Dinamap, Critikon Inc., Tampa, FL). Temperature was monitored with an intraluminal rectal probe. Arterial oxygen tension was measured with a pulse oximeter placed on the tongue. The electrical activity of the heart was followed with serial electrocardiograms (EKG) using limb lead II. Normal variability was estimated by monitoring the resting baseline state for 10 minutes and then recording the response to an injection of physiological saline. A pharmacological dose of 1.0 mg of non-radioactive Tc-99 (not 99m) TRODAT was administered intravenously through an indwelling line. Vital signs, oxygen saturation, and several EKG parameters were recorded.

Problems: Automatic inflation of the blood pressure cuff on the thigh of the animal sometimes produced leg straightening and spurious measurements of blood pressure. The animal became arousable 65 minutes after administration of TRODAT and required re-sedation with another x mg of ketamine IM.

Results: The second dose of ketamine quickly produced significant changes in several vital signs and some of the EKG parameters. However, there were no significant changes in any of these parameters with placebo or TRODAT. The rate and rhythm of the heart remained essentially constant throughout the duration of the study until the second dose of ketamine was administered. There were no acute or subacute changes in the height or width of the QRS complex. No changes could be observed in the P or the T waves. There was never any ectopy. No other signs of noradrenergically induced cardiac irritability were observed.

Conclusions: The methodology was sensitive enough to detect the pharmacological effects of ketamine. However, an effect of TRODAT could not be observed, even though the dose of TRODAT that was administered was about 25 times higher than that which will be used in humans. This suggests that TRODAT does not produce any effects of basal physiology in this dose range. No pharmacological effects should be expected in human volunteers who are studied with significantly smaller doses.

Section 9 §312.23 (9) Previous Human Experience

§312.23(9)(a) Phase I Studies

§312.23(9)(a)(i) Specific Aim: to measure the radiation dosimetry of [Tc-99m]TRODAT-1 in healthy human volunteers. The results were published by the Journal of Nuclear Medicine in 1998.

§312.23(9)(a)(ii) Introduction: This subsection begins by summarizing the studies of safety that have been performed at the University of Pennsylvania Medical Center since

the initial application was approved in March of 1997. Radiation safety is considered first, followed by a description of a clinical laboratory study of effects on internal organ function. Three analogous reports on cardiac electrophysiology and hemodynamics follow. The subsection ends by reviewing several studies in progress which suggest that TRODAT is an effective tracer for quantifying dopamine transporter levels.

§312.23(9)(a)(iv) Pharmacological Safety Study I: Studies of Effects on Internal Organ Function in Healthy Volunteers

Human subjects: The sample included 4 men and 6 women; 5 were Caucasian, 3 were African American, 1 was Hispanic, and 1 was Asian American. They had a mean age of 33.7 ±11.3 years (range:21-54). The 4 men had an average weight of 79.6 ± 7.2 kg (175 ± 16 lb.) and a mean height of 173.6 ± 4.7 cm (68.4 ± 1.9 inches). The 6 women weighed an average of 65.6 ± 1.9 kg (148 ± 17 lb.) and had a mean height of 167.5 ± 4.8 cm (65.6 ± 1.9 inches). The average education was 15.2 ± 2.4 years (range: 12-18). All the subjects were fully employed except for one man with a generally good vocational history who was temporarily between jobs.

Phlebotomy: An 18 gauge, 1.5 inch catheter was inserted into an antecubital vein and kept patent with normal saline that had been microwaved until it felt warm to the touch. Patients were then placed in a supine position on the imaging table. A 20 minute transmission scan was performed. Blood for baseline laboratory assays was drawn after the transmission scan. TRODAT was then injected through the catheter. Whole body scans were acquired over the next hour. Blood was drawn immediately before the subjects got up from the table. Blood was drawn through the same catheter to obtain the 4 hour values, but the subjects had only been supine for 20 minutes. Phlebotomy at the 24 hour mark required inserting a new needle into another vein.

Clinical assessment of effects on internal organ function: The baseline clinical laboratory tests included a complete blood cell count with differential, serum electrolytes, liver enzymes, and thyroid function tests. Levels of creatinine, blood urea nitrogen, glucose, cholesterol, triglycerides, albumin, and total protein were also assayed. The blood tests were repeated 1, 4, and 24 hours after the administration of the radiopharmaceutical. The urine tests were repeated once after 24 hours.

Clinical assessment of effects on the cardiovascular system: Electrocardiograms (EKGs) were performed. Continuous 3-lead tracings began 15-30 minutes before administration of the radiopharmaceutical. They were continued for 60 minutes after administration and then once every 10 minutes for the next hour. Discrete 12-lead tracings were obtained intermittently. A cardiologist formally reviewed the tracings. Vital signs were taken about once every 30 seconds over the same time interval.

Results: There were no subjective effects of the radiotracer on any of the volunteers other than a mild and transient burning sensation at the site of injection. The problem

was limited to the initial subjects and was probably related to the ethanol (200 μl) that the radiotracer was dissolved in. Diluting the dose in 3 ml of normal saline and reducing the rate of administration rectified the problem in the later studies. No changes in vital signs could be attributed to a drug effect. Pulse quickened in one subject who experienced urinary frequency, but it returned to baseline shortly after micturation. There were no changes noted on physical examination. Over 1,000 pages of continuous 3-lead EKG tracings did not show any meaningful changes. Serially acquired 12-lead tracing occasionally produced computer read outs of "sinus bradycardia", "sinus arrhythmia", and in two subjects, "nonspecific T-wave abnormalities". However, two cardiologists independently concluded that there were no differences between the baseline EKGs and the post administration tracings. Specifically, there were no signs of an ionotrophic drug effect similar to that expected with pharmacological doses of cocaine.

§312.23(9)(a)(v) Pharmalogical Safety Study II: [Tc-99m] TRODAT: Pharmalogical Safety in Human Research Volunteers

Purpose: To determine whether the intravenous injection of a formulation containing [Tc-99m]TRODAT-1 produces a measurable pharmacological effect on human research volunteers.

Background: TRODAT is a cocaine derivative that has been developed as a SPECT imaging agent for measuring dopamine transporters. Its Technetium-99m label and many favorable biological properties have contributed to its rapid adoption by laboratories around the world, even though few studies of its safety have been published.

Methods: The sample consisted of 100 healthy human volunteers aged 18 to 83. They were placed in a supine position on an imaging table for at least 20 minutes before and after injection. Vital signs and 12 lead electrocardiograms (EKGs) were acquired at 5 minute intervals. Each 740 MBq (20 mCi) dose contained between 0.067 to 0.13 mg of radiolabeled plus unlabeled TRODAT, and was administered by a single investigator with a uniform technique through an indwelling catheter that had been in place for at least 20 minutes.

Results: During the first 5 minutes after injection, the systolic blood pressure rose from 122.7 ± 18 to 124.6 ± 20 mm Hg ($p = 0.017$) and the diastolic blood pressure increased from 68.6 ± 10.4 to 70.4 ± 9.8 mm Hg ($p = 0.0024$). Heart rate dropped continuously throughout the study. The group mean change from 63.6 to 62.5 ± 10 beats per minute during the first 5 minutes after injection was significant ($p = 0.034$), but clinically silent. The QT and QTc intervals increased continuously throughout the study except during the first 5 minutes after injection. Otherwise, there were no detectable drug effects on the EKG tracings. There were no other side effects associated with receiving the tracer.

Conclusion: Injecting TRODAT produces a statistically significant hemodynamic effect, which in some ways resembles the peripheral, but not the central, effects of cocaine. However, the effect is too small to detect without large samples sizes. It does not appear to be clinically meaningful. It follows that TRODAT is probably a safe

radiopharmaceutical for imaging dopamine transporters when administered in this dose range.

§312.23(9)(a)(vi) Pharmacological Safety III: Co-Administering Methylphenidate and TRODAT to Human Research Volunteers

Introduction: To determine if the co-administration of TRODAT and other medications that block the dopamine transporter is safe and effective.

Backround: TRODAT is a cocaine derivative that has been developed as a SPECT imaging agent for non-invasively measuring dopamine transporter levels with SPECT techniques. Studies in 100 healthy human volunteers showed that it produced hemodynamic effects that resemble the actions of cocaine. However, when given alone, the effect size of less than 2% is too small to detect without very large sample sizes, and seems to be clinically meaningless. An identical study of 178 patients with parkinsonian movement disorders produced virtually identical results. As importantly, no additional drug interactions were detected with the dopaminergic medications that are prescribed to treat the disorder. However, none of these medications block the dopamine transporter. This study investigated the safety of administering TRODAT when a drug with a similar mode of action was already on board in pharmacologically active doses.

Methods: The within subjects design included 8 healthy human volunteers aged 18 to 41. They were administered either 10 mg of methylphenidate (Ritalin) or 325 mg of aspirin mixed in fruit juice. About a half hour later, they were placed in a supine position on an imaging table for at least 20 minutes before and after injection of TRODAT at exactly 60 minutes after methylphenidate administration. Vital signs and 12 lead electrocardiograms (EKGs) were acquired at 5 minute intervals. SPECT scans of the brain were acquired from 3 to 4 hours after injection. Neuropsychological tests were given during the uptake interval. The scans were analyzed automatically with statistical parametric mapping (SPM) techniques as well as manually.

Results: The resting heart rates were lower while on methylphenidate than on placebo at every time point (69.0 ± 12 v. 68.3 ± 12, p=0.031). During the first 5 minutes after injection, the systolic blood pressure rose from 122.7 ± 18 to 124.6 ± 20 mm Hg (p = 0.017) and the diastolic blood pressure increased from 68.6 ± 10.4 to 70.4 ± 9.8 mm Hg (p = 0.0024). Heart rate dropped continuously throughout the study. The group mean change from 63.6 to 62.5 ± 10 beats per minute during the first 5 minutes after injection was statistically, but not clinically, significant (p = 0.034). The QT and QTc intervals increased continuously during the study except during the first 5 minutes after injection. Otherwise, there were no drug effects on the EKG tracings of any kind. Subjectively, methylphenidate tended to elevate mood and increase vigor. It had a visibly pronounced effect on the uptake of TRODAT in all subjects. SPM analysis showed that the effect was highly significant in all subregions of the basal ganglia. Quantification showed that drug occupancy reduced the specific uptake of TRODAT by an average of 44 ± 9% (range: 29 to 60%). Drug induced changes on all neuropsychological performance scores were highly variable, with no group means approaching significance. However, reductions in

TRODAT uptake values by drug occupancy were positively correlated with drug induced changes in verbal learning, approaching significance in the caudate (r=0.46), but not the putamen (r=0.20). Drug induced impairment in complex motor coordination was correlated with increased drug occupancy on the non-dominant side (r= -0.92), but not the dominant side (r=0.02). There were no relationships with motor speed. Changes in mood were more related to blockade in the caudate (r=0.67) than the putamen (r=0.18).

Conclusion: Administering psychoactive doses of methylphenidate by mouth significantly lowers heart rate and increases blood pressure. Even though the magnitude of the changes average less than 5%, the profile is typical of the effects produced by cocaine-like drugs on hemodynamics. Co-injecting TRODAT 1 hour after the administration of methylphenidate does not appear to produce any further changes in hemodynamics. It follows that TRODAT is a safe radiopharmaceutical for imaging dopamine transporters when administered in this dose range.

§312.23(9)(a)(vii) Pharmalogical Safety Study IV: Administering TRODAT to patients with movement disorders:

Purpose: To determine whether the intravenous injection of a formulation containing [Tc-99m]TRODAT-1 produces a measurable pharmacological effect on patients with movement disorders.

Background: TRODAT is a cocaine derivative that has been developed as a SPECT imaging agent for measuring dopamine transporters. Patients with movement disorders may be one of the primary beneficiaries because its Technetium-99m label and many favorable biological properties have contributed to its rapid adoption by laboratories around the world, even though few studies of its safety have been published.

Methods: The sample consisted of 194 patients with parkinsonian movement disorders aged 23 to 84. They were placed in a supine position on an imaging table for at least 20 minutes before and after injection. Vital signs and 12 lead electrocardiograms (EKGs) were acquired at 5 minute intervals. Each 740 MBq (20 mCi) dose contained between 0.067 to 0.13 mg of radiolabeled plus unlabeled TRODAT, and was administered by a single investigator with a uniform technique through an indwelling catheter that had been in place for at least 20 minutes.

Results: A single patient had a shift in the site of her atrial pacer and went into a sinus rhythm of 120 BPM starting 20 minutes after administration which lasted for less than 1 minute. About 10% of the patients reported perceiving the smell of alcohol starting about 10 seconds after injection and lasting 3-5 seconds. Otherwise, there were no observable effects within any given subject. During the first 5 minutes after injection, blood pressure increased slightly in 58.6% of the subjects and diastolic rose in 47.4%. The systolic blood pressure increased from a group mean of 133.5 to 137.9 ± 22 mm Hg ($p < 10EE-9$) and the diastolic blood pressure increased from 73.6 to 75.1 ± 9 mm Hg ($p < 0.00002$). Heart rate dropped continuously throughout the study. The group mean decreased from 68.8 to 68.2 ± 12 beats per minute during the first 5 minutes after injection ($p = 0.024$). The QT

and QTc intervals increased continuously throughout the study. Otherwise, there were no detectable drug effects on the EKG tracings.

Conclusion: Injecting this formulation containing TRODAT into patients with movement disorders produces the same effects on hemodynamics that it does in healthy humans. In some ways, the profile resembles the peripheral, but not the central, effects of cocaine. However, the effect is too small to detect without large sample sizes. It does not appear to be clinically meaningful in most patients. It follows that TRODAT is probably a safe radiopharmaceutical for imaging dopamine transporters when administered in this dose range.